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Tissue microarray: Current perspectives in pathology

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Tissue microarray technology involves core needle biopsies of multiple tissues constructed in the same block. It is a new method used to analyze several hundred tissues, especially tumor samples at a single slide. It may be prepared from archival formalin-fixed, paraffin-embedded tissues or from fresh tissues by cryoarray. One difficulty with paraffin-embedded tissues is due to antigenic changes in proteins and mRNA degradation induced by the fixation and embedding processes. But it is shown that many proteins retain their antigenity for more than 60 years. Since it provides to study a parameter for 100-1000 samples on a single slide, community based retrospective cohort studies will be available. The equipments are commercially available for microarray technique. Predicting the response of chemotherapeutics, comparison of methods, testing interlaboratory and interobserver reproducibility of methods are easier, cheaper and faster with tissue microarray technology.

Keywords: tissue microarray, cryoarray

Introduction

Tissue microarray technology (TMA) has been used, discussed or described in more than 250 scientific publications. It involves core needle biopsies of multiple pre-existing paraffin-embedded tissue blocks and re-embedding them in the form of an arrayed master block. Thus it means biopsy of a biopsy. Construction of multiple tissue arrayed block is also possible by fresh tissues and frozen section, as well.[1]

The first reports concerning TMA appeared through 1998–2001 by Kannonen, Kallioniemi et al from National Human Genome Research Institute, Bethesda, USA[2–8] They worked on many different tumor types, about the technique and different markers. After that so many papers about this subject has become appearing in literature (Table 1).

Tissue microarray technology is a new method used to analyze several tissues especially tumor samples on a single slide.[1] The recent development of tissue microarray technology has potentiated large-scale retrospective cohort studies using archival formalin-fixed, paraffin-embedded tissues.[9] It is shown that many proteins retain their antigenicity for more than 60 years.[10] A major obstacle to broad acceptance of microarrays is that they reduce the amount of tissue analyzed from a whole tissue section to a disk, 0.6 mm in diameter.[11] As many as 1000 cylindrical tissue biopsies from individual tumors can be distributed in a single tissue microarray. Sections of the microarray provide targets for parallel in situ detection of DNA, RNA and protein targets in each specimen on the array, and consecutive sections allow the rapid analysis of hundreds of molecular markers in the same set of specimens.[2] One difficulty with paraffin-embedded tissue relates to antigenic changes in proteins and mRNA degradation induced by the fixation and embedding process.[1] But there are technical reports to improve preservation of genome DNA and proteins in paraffin blocks, such as zinc based fixation, buffered formalin fixation.[12] There are reports describing construction and use of frozen arrays.

Analysis of hundreds of specimens from patients in different stages of disease is needed to establish the

diagnostic, prognostic and therapeutic importance of each of the emerging cancer gene candidates.[13–18] Most of the applications of the TMA technology have come from the field of cancer research. Examples include analysis of the frequency of molecular alterations in large tumor materials, exploration of tumor progression, and identification of predictive or prognostic factors and validation of newly discovered genes as diagnostic and therapeutic targets.[3] It can be used to correlate lymph node positive and negative tumors, it helps for molecular classification of tumors. It provides rapid linking of molecular changes to clinical endpoints. Predicting the response of chemotherapeutics or hormonotherapy[19,20], comparison of methods, testing interlaboratory and interobserver reproducibility of methods is easier and faster with TMA. Since it provides studying a parameter for 100-1000 samples on a single slide, community based retrospective cohort studies could be available.[21] By this way the tissue microarray data exchange specification: a community based open source tool for sharing tissue microarray data collection is needed.

TMA techniques

There are two types of TMA technique, automated and manual. In automated method you can mark, edit and save punch coordinates using an on-screen display and software tools, while you can perform visual selection during punching, using magnifying glass or a stereomicroscope as a guide. You are faster in the

Table 1. The distribution of studies in the literature about tissue microarray.

Cancer Type	Topics	% of manuscripts
Breast carcinoma	Estrogen, progesterone, Her2/neu, telomerase, cyclin D1, erbB2, BRCA1-2, mamoglobin, snoN, KIT, MCSF-1, minichromosome	18.4
Skin and malignant melanoma	Tirozin kinase, CD117, Act trans factor	17.6
Prostate carcinoma	CD10, PTEN, NCAM, Sindecan	14.4
Lymphoma/leucemia	Cyclin E, CD20, Pax 5, P18IN4C, CD44, SHP	5.5
Transitional carcinoma	FGFs, EMS1, erbB2, Her2/neu	5.5
Colorectal carcinoma	P 53, Mismatch repair, c-myc	4.7
Renal cell carcinoma	Keratin, steroid hormones, muc-1, cyclins, p53, VEGF	4.5
Lung Carcinoma	EGF, e-cadherin, her2/neu	4.5
Gastric carcinoma	Gastrin, cyclooxigenase 2,c-met	3.6
Ovarium	Erbb2, p53, A103, HIF	2.8
Hepatic tumors	P53, vimentin, hepatitis B	2.8
Endometrium	STK15, mismatch repair	1.2
Testis tumors	NKX3-1, Oct 3 / 4	1.2
Surrenal tumors	A103, Chromogranin	0.8
Review articles		3.2
Technical reports		6.7
Others	Tiroid, paratiroid, Synovial sarcoma, osteosarcoma, MFH, histiositosis	2.4

automated method in punch speed and the block capacity is 7 times more than manual method. Video-merge unit displays pre-marked slide images side-by-side to the donor block image in the automated method, while pathologist marks regions of interest to slides by hand before arraying in manual method. Punch sets of 0.6 mm 1.0 mm 1.5 mm 2.0 mm are available. Automated Tissue Microarrayers, Manual Tissue Arrayers are also commercially available. Automated evaluation is also possible with a DNA microarray scanner.

TMA construction can be summarized as follows[22, 23]:

1. Formalin-fixed and paraffin-embedded tissues are subjected to routine sectioning of 3-5 μm thickness and HE staining.
2. The typical tumor spots are chosen under microscopy for each case and marked on the corresponding spot on the tissue block.
3. Then, cylindrical tissue columns were punctured with tissue arrayer (Beecher Instruments, USA; China patent no. 03113734.2[22]) in the marked area, or one can use any punch biopsy needle.
4. They are transferred to corresponding receiver pore of the prepared block manual; with a special paraffin-fixing box (China patent no. 03113733.4)[22]; or by automated arrayer (Beecher Instruments, USA).
5. The tissue array block is then completed according to the predetermined scheme.
6. The block is heated at 40 °C for 15 minutes and the surface was flattened for subsequent section of 5 μm thickness.[23]

A sample of manual constructed TMA in our laboratory is shown in Figure 1.

Cryoarray construction can be summarized as follows[24]:

1. A standard 37x24 mm plastic mold is filled with liquid optimal cutting temperature compound.
2. A specially designed cryoapparatus carrying 48 pins of 3 mm diameter is placed in the cryomold.
3. Solidified at –80 ºC, the cryoapparatus is removed.
4. A specified core biopsy needle in 3 mm diameter is used to punch tissues and transferred to the recipient block
5. Serial sections are taken and the searched procedure is applied.

Conclusion

A full understanding of the molecular genetics and signaling pathways involved in cancer development and in the metastatic process is of central importance for developing innovative and novel treatment options. The most common tumor type studied by TMA is breast carcinoma. The distribution of subjects studied by TMA is shown at Table 1.

As TMA induces the amount of tissue required, it will be important when amounts of tissue available are limited. An important problem is; as it reduces the amount of tissue available for analysis, the technique may not be representative of antigen expression patterns across a whole tumor. The adequate number of disks required to represent the expression of common antigens in common tumors has been started to be evaluated[9] It must be evaluated for each tumor type, especially in polymorphic tumors.

Autopsy evaluation could be easier, faster and cheaper with more tissue types examined. But number of minimum cores required per each organ according to the ages should be determined comparing with routine sections. RNA in situ hybridization is possible by cryoarrays that means TMA using frozen tissues.[25] TMA with validation on full tissue sections is much useful for each tumor type.[26] Quality control is needed. Reference tissues must be used as control and guide. The tissue shame in order and coordinates should be well documented and archived.

Non tumoral studies are rare. These are about normal tissues, renal transplantation[27], and about cerebral tissues and autism.[28] The most common used method is immunohistochemistry; fluorescence in situ hybridization is also available.[29] Briefly all techniques that can be performed on paraffin embedded tissues are also available on TMA. Quantitative analyses are also possible on TMA tissues.[30] This method may be useful in studying the laboratory animal tissues much easier and cheaper. All tissues will be studied from all animals.

TMA technology will be widely used in pathology practice and research by its perspective of economy.[31] TMA studies will fasten the decision of which molecular biomarkers would achieve acceptance in the clinical setting. Studying the archival paraffin blocks of patients with a well known survival and

outcome seems to be more useful and reliable than gene microarrays since the researcher observes the tissues on slides. Nowadays, gene microarray studies gain acceptance when they are confirmed by other molecular techniques. TMA studies if depended on reliable knowledge about the representation of the tissue will be very useful in pathology research and practice.

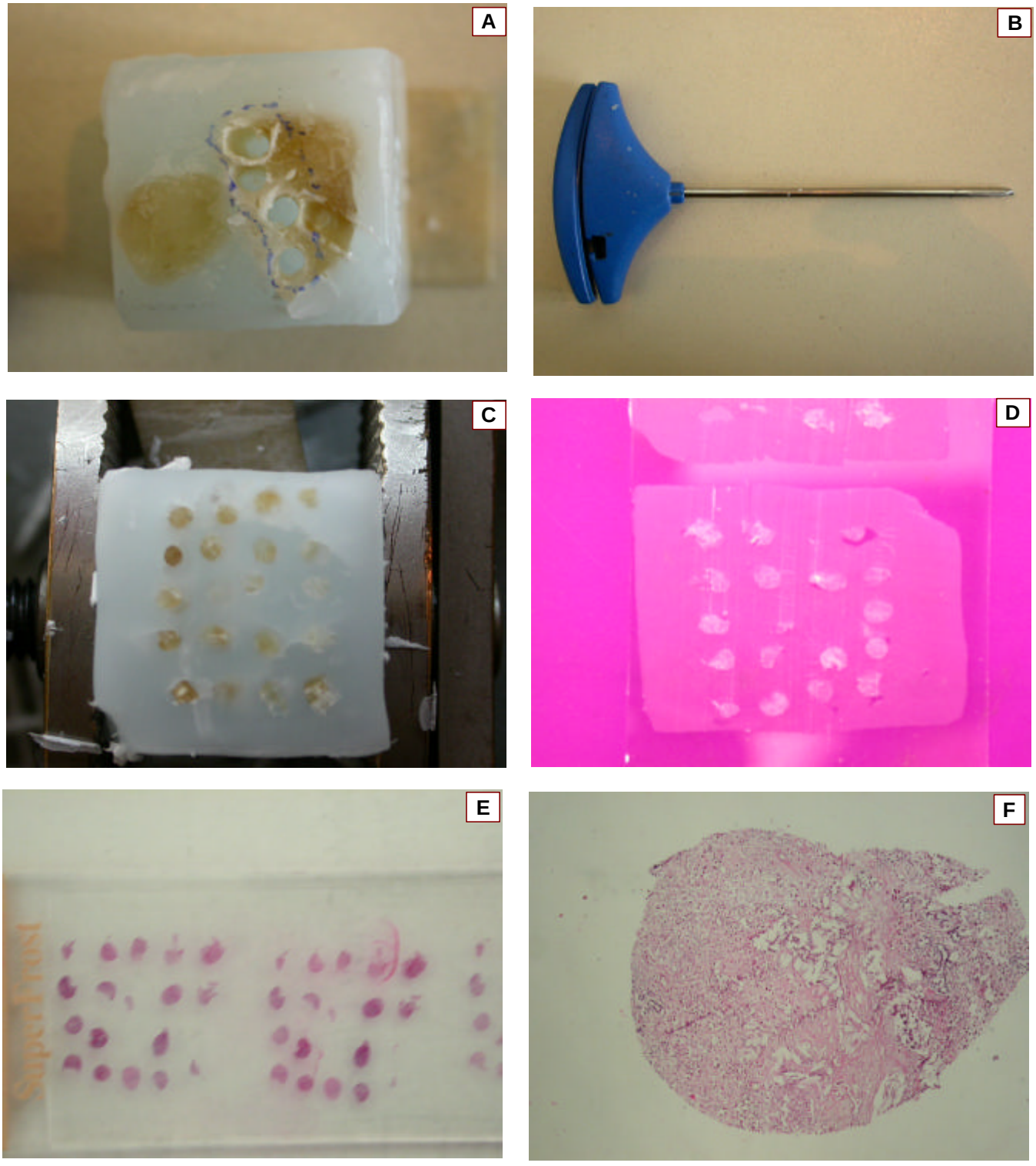

Figure1: *Please see legend on the next page*

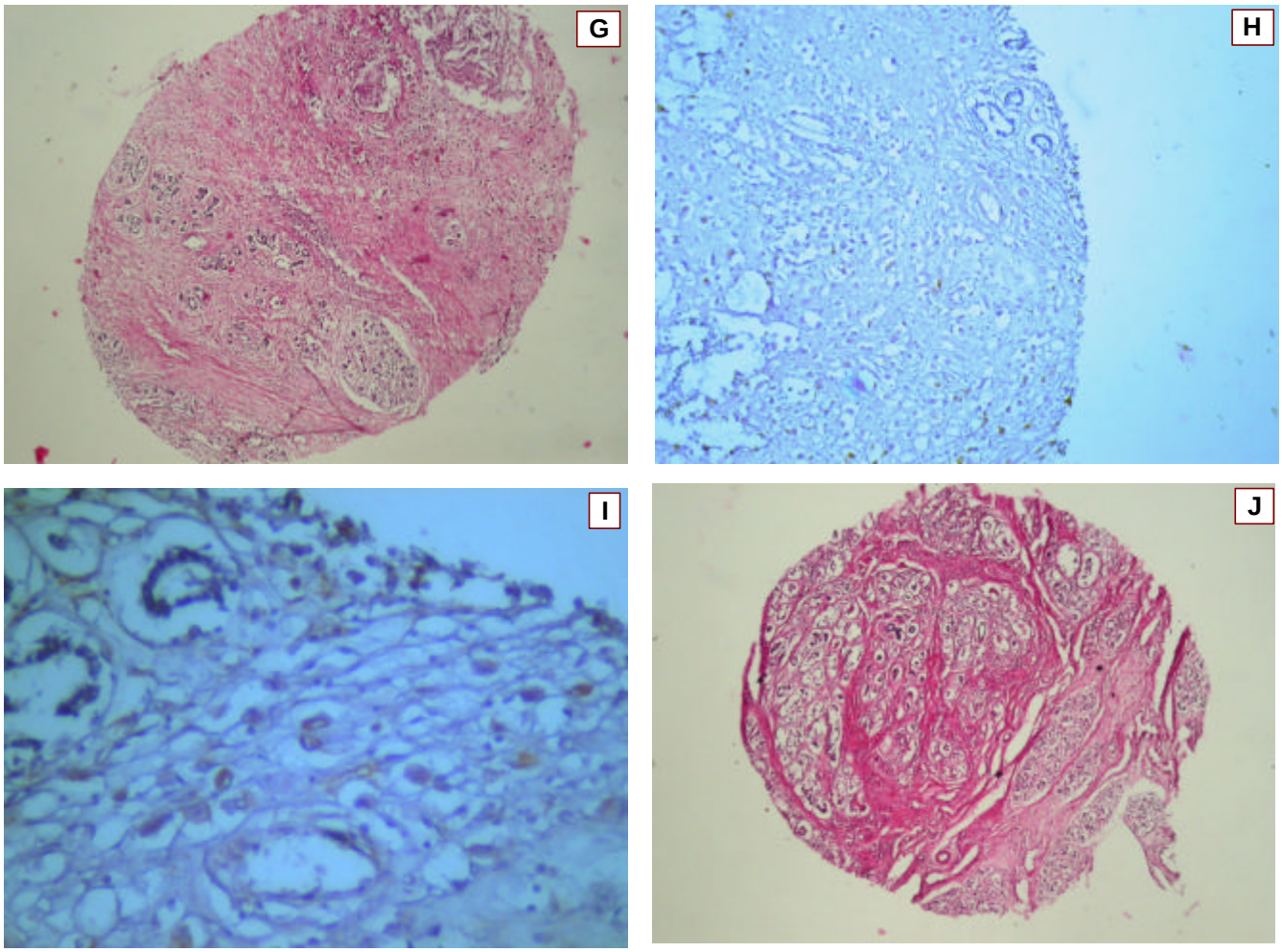

Figure1: Construction of manual tissue microarray (Dr Behçet Uz Children's Research Hospital, Pathology Laboratory) *(continued from the previous page)*
A: Selection of tissue area from the paraffin block
B: The needle used to micro biopsy from the block
C: New constructed paraffin block
D: 5 micrometer section on the slide
E: H&E stained section
F,G,H, I, J: Samples from the H&E stained slide

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